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Novel cell-binding activity specific for *N*-acetyl-D-glucosamine in an *Escherichia coli* strain

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Escherichia coli strains isolated from patients with different levels of urinary tract infection and from healthy persons were tested for their ability to haemagglutinate endo-β-galactosidase-treated human erythrocytes. Among the 104 strains studied one revealed a strong agglutination reaction with the enzyme-treated erythrocytes. From the monosaccharides tested *N*-acetyl-D-glucosamine inhibited agglutination most effectively. Orosomucoid and asialo-orosomucoid had no effect on the haemagglutination whereas β-galactosidase treated asialo-orosomucoid was inhibitory. These findings indicate that the *E. coli* strain studied contains a novel cell-binding activity with specificity for terminal *N*-acetyl-D-glucosamine residues.

N-*Acetyl-D-glucosamine* *Bacterial adhesion* *Haemagglutination* *Endo-β-galactosidase* *Receptor* *Erythrocyte*

1. INTRODUCTION

Bacterial adhesion to host epithelium is an important virulence factor in many infectious diseases [1], and has probably also a role in the stabilization of the normal bacterial flora; e.g., in the mouth and intestine [2,3]. It is evident that this adhesion is receptor-mediated, although only a few receptors are known at the molecular level. Most *Escherichia coli* strains, regardless of their origin, are able to bind to α-D-mannosyl residues on epithelial cells and erythrocytes by their type I fimbriae [4–6]. Most *E. coli* strains associated with human pyelonephritis carry P fimbriae, which recognize blood-group P determinants on human erythrocytes and urinary tract epithelial cells [7–9]. Two other receptor structures for *E. coli* are also known: blood-group M determinants [10] and sialyl (α2–3)galactosides [11]. The molecular nature of the bacterial adhesins binding to the latter two structures has not yet been resolved. We now describe a novel binding specificity in a strain of *E. coli*. This interaction is based on the bacterial binding to *N*-acetyl-D-glucosamine residues that become exposed after treating erythrocytes with endo-β-galactosidase.

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2. MATERIALS AND METHODS

2.1. *Bacteria*

Three *E. coli* strains showing mannose-resistant blood-group M-specific haemagglutination were studied: IH 11165 (serotype 02) was isolated from a patient with acute pyelonephritis [10], IH 3082 (08H19) from a patient with newborn meningitis, and KS 300 was kindly donated by Dr G. Källenius (National Bacteriological Laboratory, Stockholm) and was not typable with the antisera available [9]. In addition, 63 strains lacking D-mannose-resistant haemagglutination activity of human OP_1 or $O\overline{p}$ erythrocytes, and 38 strains showing only P-specific agglutination, were tested. These strains had been isolated from the urine of patients with different levels of urinary tract infection (85 strains) or from faeces of healthy children (16 strains). The strains and their serotyping have been

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described [9]; (submitted). The strains lacking D-mannose-resistant haemagglutination were of O-serotypes: 1 (four strains), 2 (three strains), 4 (two strains), 6 (twelve strains), 7 (one strain), 8 (five strains), 9 (two strains), 18 (five strains), 22 (seven strains), 25 (six strains), 50 (three strains), 75 (seven strains), 77 (five strains) or 85 (one strain). The P-fimbriated strains were of O-serotypes: 1 (seven strains), 2 (seven strains), 4 (nine strains), 6 (five strains), 18 (four strains), 22 (one strain), 75 (one strain) or 16 (three strains). One of the P-fimbriated strains was not typable with the sera available. For haemagglutination tests the bacteria were grown on CFA-agar plates as in [9].

2.2. *Endo-β-galactosidase treatment*

Washed human erythrocytes (250 μl) were suspended in 650 μl 10 mM sodium phosphate (pH 7.4) containing 0.15 M NaCl, and 10 mU *Escherichia freundii* endo-β-galactosidase (Seikagaku Kogyo, Tokyo) was added in 100 μl 50 mM sodium acetate buffer (pH 5.8). The erythrocytes were incubated at 37°C for 2 h with occasional mixing and washed by repeated centrifugations.

The appearance of terminal *N*-acetylglucosamine residues on the erythrocytes was monitored by incubations with galactosyltransferase and UDP-D-[^{14}C]galactose, which indicated the presence of 2.5×10^6 acceptor sites/cell in the treated erythrocytes as opposed to 0.05×10^6 sites/cell in untreated erythrocytes (in preparation).

2.3. *Haemagglutination tests*

Haemagglutinations were performed on glass slides at room temperature as in [6,9,11]. All haemagglutinations were done in the presence of 5% (w/v) methyl α-D-mannoside to study only D-mannose-resistant agglutination. For haemagglutination inhibition studies the suspensions of *E. coli* IH 11165 (about 10^{10} bacteria/ml 10 mM sodium phosphate buffer (pH 7.1), 0.15 M NaCl) were titrated in 2-fold dilutions with the erythrocytes, and a bacterial concentration corresponding to twice the lowest haemagglutinating concentration was used.

2.4. *Inhibitors*

All monosaccharides and glycoproteins used were dissolved in 10 mM sodium phosphate containing 0.15 M NaCl and adjusted to pH 7.1. Orosomucoid was kindly supplied by Dr G. Myllylä (Finnish Red Cross Blood Transfusion Service, Helsinki) and desialylated by treatment with 0.1 M HCl at 80°C for 1 h followed by dialysis against 50 mM NaCl; 20 mg desialylated orosomucoid was incubated in 2 ml 25 mM sodium citrate buffer (pH 3.8) containing 10 μl toluene and 5 U purified *Aspergillus niger* β-galactosidase (kindly supplied by Dr J. Knowles, Biotechnical Laboratory, Technical Research Center of Finland) at 37°C for 3 days, after which 5 U enzyme was added and the incubation continued for 2 days during which time no more D-galactose was released. Gas–liquid chromatographic analysis [12] of the dialysed sample indicated that 55% of D-galactose residues had been removed.

3. RESULTS AND DISCUSSION

To prevent haemagglutination reactions due to the D-mannoside-binding activity commonly present in different strains of *E. coli* [4–6], all agglutination studies were performed in the presence of methyl α-D-mannoside. For the detection of binding activities which under these conditions do not induce haemagglutination, we investigated whether erythrocytes could be modified by endo-β-galactosidase to render them agglutinable.

It is known that human erythrocytes contain only small amounts of terminal *N*-acetyl-D-glucosamine residues ([13], in preparation). On the other hand, endo-β-galactosidase is known to specifically cleave the polyglycosyl chains [14] present in the erythrocyte membrane glycoproteins and glycolipids so that terminal *N*-acetyl-D-glucosamine residues become exposed [15,16]. We therefore tested 65 non-haemagglutinating strains of *E. coli* using endo-β-galactosidase treated P_i erythrocytes to reveal *N*-acetyl-D-glucosamine recognition. In addition 38 strains known to contain blood-group P-specific adhesion activity [7–9] were tested with endo-β-galactosidase treated $\bar{p}$ erythrocytes and 3 strains known to contain blood-group M-binding specificity [10] were tested with enzyme-treated NN erythrocytes to avoid haemagglutinations due to these binding specificities.

From the 104 *E. coli* strains studied, one (IH

11165) revealed a strong agglutination reaction with endo-β-galactosidase treated erythrocytes (table 1). This strain has previously been shown to contain a binding activity with specificity for blood-group M erythrocytes [10]. These two specificities seem, however, to be clearly distinguishable, since the two other strains containing the M-specific activity did not agglutinate enzyme-treated N erythrocytes (table 1). Endo-β-galactosidase treatment had only a slight effect on the agglutination of M erythrocytes by these strains. Expectedly, there was a slight increase in the titer of strain IH 11165, as opposed to a slight decrease in the titer of the two other strains.

To study whether the haemagglutination of the endo-β-galactosidase treated erythrocytes by strain IH 11165 was in fact due to recognition of *N*-acetyl-D-glucosamine residues, different monosaccharides were tested for haemagglutination inhibition. *N*-Acetyl-D-glucosamine inhibited the haemagglutination at 2.1 mM, D-glucose and D-mannose were much less effective, and *N*-acetyl-D-galactosamine, L-galactose and L-fucose were ineffective at the concentrations studied.

In accordance with the haemagglutination inhibition studies with the monosaccharides, the glycoprotein orosomucoid and its desialylated derivative did not cause haemagglutination inhibition at the concentrations studied (1.9 mg/ml, table 1). However, β-galactosidase-treated orosomucoid inhibited haemagglutination at about 0.5 mg/ml, which corresponds to about 0.13 mM with respect to terminal *N*-acetyl-D-glucosamine residues. The concentrations of monosaccharide and macromolecular inhibitors were thus of the same orders of magnitude as those required to inhibit D-mannoside or neuraminyl(α2–3)galactoside recognition [11,17].

Table 1

Agglutination titers of *E. coli* strains with control and endo-β-galactosidase-treated erythrocytes

Erythrocytes	*E. coli* strain		
	IH 11165	IH 3082	KS 300
MM	256	512	256
MM + endo-β-galactosidase	512	64	128
NN	0	0	0
NN + endo-β-galactosidase	64	0	0

Table 2

Inhibition of haemagglutination of endo-β-galactosidase-treated erythrocytes by *E. coli* IH 11165

Inhibitor	MIC[a]
N-Acetyl-D-glucosamine	2.1 mM
D-Glucose	32 mM
D-Mannose	130 mM
L-Fucose	>130 mM[b]
D-Galactose	>130 mM[b]
N-Acetyl-D-galactosamine	>130 mM[b]
Orosomucoid	>1.9 mg/ml
Asialo-orosomucoid	>1.9 mg/ml
Agalactosyl-orosomucoid	0.48 mg/ml

[a] Minimal inhibitory concentration required to prevent haemagglutination
[b] Not inhibitory at 130 mM

From the *E. coli* strains studied those classified as non-haemagglutinating were faecal isolates from healthy persons and urinary isolates from patients with different levels of urinary tract infection. The P-fimbriated strains were mostly pyelonephritogenic and represented all the common serotypes of *E. coli* strains associated with human pyelonephritis. Thus, although strain IH 11165 was isolated from a 1-year-old girl with acute pyelonephritis [9,10], it seems that the novel binding specificity is rare at least among human pyelonephritogenic strains.

The molecular nature of the bacterial haemagglutinin with specificity for *N*-acetyl-D-glucosamine is so far unresolved. We have found that the purified *E. coli* IH 11165 fimbriae show a strong M-specific haemagglutination but lack the *N*-acetyl-D-glucosamine binding activity (unpublished). It may be that *N*-acetyl-D-glucosamine recognition resides in a non-fimbrial haemagglutinin which has been proposed to exist in some *E. coli* [18]. Such haemagglutinins have not been purified and their molecular nature is so far unknown.

An important aspect of the findings here is also that the binding property of a bacterial strain can only be observed after modification of the erythrocyte surface; e.g., by the endo-β-galactosidase used here. It is known that some fimbriae, such as the

987P fimbriae of porcine enterotoxigenic *E. coli* [19], have no demonstrable haemagglutination activities, although they mediate adhesion to epithelial cells. A likely explanation is that the appropriate receptor structures are lacking on the erythrocyte surface. Use of specific enzymes, such as glycosidases and glycosyltransferases, to modify the erythrocyte surface structures could be a useful tool in the identification and characterization of microbial receptors.

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